Papillomatosis in chaffinches (*Fringilla coelebs*) in the Czech Republic and Germany

I. Literák[1], B. Šmíd[2], L. Valíček[2]

[1]Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic

ABSTRACT: Analyses of ornithological field records, direct observations of typical lesions, and examination by electron microscopy revealed leg papillomatosis in six chaffinches (*Fringilla coelebs*) in the Czech Republic and one chaffinch in Germany. Papillomavirus was identified by electron microscopy using the negative staining technique in skin lesions of one chaffinch caught in the Czech Republic. This is the first report of papillomatosis in chaffinches recorded in the Czech Republic.

Keywords: papilloma; virus; bird; cutaneous lesions; disease

Papillomaviruses are strictly host specific DNA viruses that induce development of mostly benign, but occasionally malignant, neoplastic lesions in mammals and birds. Compared with mammalian infections, reports on avian papillomatosis are rather scarce (Sironi and Gallazzi, 1992). Most of them described leg and feet papillomas in chaffinches (*Fringilla coelebs*) (Lina, 1970; Lina *et al.*, 1973) in which papillomavirus-like particles were identified by electron microscopy. The isolates were later characterised, cloned and classified with the genus *Papillomavirus*, and designated FPV (Fringilla Papillomavirus) (Osterhaus *et al.*, 1977; Moreno-Lopez *et al.*, 1984). Papillomas induced apparently by the same agent were observed rather exceptionally also in the related species brambling (*Fringilla montifringilla*) (Lina *et al.*, 1973). Further papillomavirus-induced lesions were described in an African grey parrot (*Psittacus erithacus*) (O'Banion *et al.*, 1992) and during outbreaks of papillomatosis in captive water birds (Zangger and Müller, 1990) and captive greenfinches (*Carduelis chloris*) (Sironi and Gallazzi, 1992).

The aim of this report is to describe cases of infectious leg papillomatosis in chaffinches observed recently in the Czech Republic and in Germany.

MATERIAL AND METHODS

Observation

Data for this report were obtained from retrospective analyses of field records, photos and video recordings showing papillomatous lesions in chaffinches originating from six sites in the Czech Republic and one site in Germany. The material was collected during bird ringing activities.

Demonstration of papillomavirus

Tissue samples collected from one skin lesion of a dead chaffinch were prepared for electron microscopy by the negative staining technique. Small tissue pieces (approximately 0.5 mm^3) were lysed in several drops of distilled water and a grid coated with a formvar film and carbon was put onto the surface of the lysate. After 10 to 15 s, the grid was removed and residual water was sucked off from its surface with a strip of filter paper. The preparation was treated with one drop of 2% water solution of ammonium molybdate for several seconds and, after sucking the excess stain off, it

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. 161 700 001) and the Ministry of Agriculture of the Czech Republic (Grant No. MO3-99-01).

was ready for viewing in the electron microscope Tesla BS 500.

RESULTS

Observation

The papillomatous lesions seen in the chaffinches corresponded to descriptions of lesions induced by the papillomavirus FPV (Groth and Abs, 1967; Lina *et al.*, 1973, for others see Table 1). In all cases, the lesions were typical of papillomas induced by FPV.

Czech Republic

1. Retrospective checks of J. Škopek's field records provided information on findings of papillomatous lesions in two 2-year-old female chaffinches netted for ringing on August 8 and 12, 1982, respectively, in

Table 1. Survey of papilloma findings in chaffinch (*Fringilla coelebs*)

Country	No. of cases, season	Lesions, diagnosis	Reference
Great Britain	1	papilloma claw	Jennings, 1959
Great Britain	2	papillomas on feet	Washington, 1964
Great Britain	8	large papillomas on feet, wart-like growths	Keymer and Blackmore, 1964
Germany	1	papilloma on foot	Keymer and Blackmore, 1964
Great Britain	6 $2^1/125^2$	papillomas on feet	Macdonald, 1965
Germany	1	papilloma on foot	Macdonald, 1965
Germany	19 (December 1963, November, December 1964)	papillomas on feet	Groth and Abs, 1967
Great Britain	16	squamous papillomas of the feet, H (in 8 cases)	Blackmore and Keymer, 1969
Great Britain	$10^1/20^2$	a scaly wart-like growth of the legs and toes	Blackmore and Keymer, 1969
Great Britain	$4^1/244^2$ (prevalence 1.6%)	papillomatous conditions of the feet	Blackmore and Keymer, 1969
The Netherlands	1 (September 1965)	wart-like excrescences on foot	Lina, 1969
The Netherlands	1 (24 October 1965)	wart-like excrescences on foot	Lina, 1969
The Netherlands	1 (30 October 1968)	papilloma on foot, H	Lina, 1969
The Netherlands	1 (22 November 1969)	papilloma on foot, EM particles 37.5 nm	Lina, 1970
The Netherlands	$330^1/25\ 000^2$	wart-like excrescences on foot	Lina *et al.*, 1973
	77 specimens	papillomas, H	
	4 specimens	EM particles 37.5 nm	
The Netherlands	1	papilloma on foot, virus isolation	Osterhaus *et al.*, 1977
Sweden	1	warts on foot, virus isolation	Moreno-Lopez *et al.*, 1984
Germany	1	papilloma on foot	this study
Czech Republic	6	papillomas on feet, EM in 1 case	this study

H = histological proof; EM = electron microscopy
[1]positive; [2]examined

the village Sudslavice (district of Prachatice, South Bohemia, 49.05 N 13.48 E). The lesions were found on the left leg in one and on the right leg in the other bird. The size of the lesion was larger in the first bird being approximately 1 cm in diameter. As stated by J. Škopek, the morphology of the lesions corresponded to the photo in the paper by Lina *et al.* (1973) and to Figures 1 and 2. Another 69 unaffected chaffinches were netted at the same site in August 1982.

2. A chaffinch with a right leg tumour was observed and recorded with a video camera by M. Strnad near a feeding box in the village Chýnov (district of Tábor, South Bohemia, 49.24 N, 18.48 E) on December 6, 1998. A comparison of the video recording with Figures 1 and 2 showed that the tumour type corresponded to a papilloma. Its size was between 5 and 10 mm. The bird kept one leg permanently retracted in plumage and used it only sporadically to gain balance.

3. One male bird of the four chaffinches netted by V. Kovář during a ringing activity near a feeding box in the village Dvorec close to Nepomuk (district of Pilsen-South, Southwestern Bohemia, 49.31 N, 13.36 E) on November 24, 2001 showed marked papillomatous leg lesions (Figure 1). All the four birds were released after ringing.

4. An adult male chaffinch showing papillomatous lesions on the left leg (Figure 2) was netted by I. Literák and his students during a bird ringing activity near a feeding box in a town park in Brno (41.13 N, 16.36 E) on December 9, 2001. The bird was caged and died several days later. The dead body was frozen to –18°C and samples of the papilloma lesions were examined by electron microscopy several weeks later. Further six chaffinches free of papillomas were netted during the same day of ringing.

Figure 1. Leg papilloma in a chaffinch (*Fringilla coelebs*); November 24, 2001, Dvorec close to Nepomuk, Czech Republic. Photographed by V. Kovář

Figure 2. Leg papilloma in a chaffinch (*Fringilla coelebs*); December 9, 2001, Brno, Czech Republic. Photographed by L. Bárta

5. An adult chaffinch with papillomatous lesions on a leg was netted by J. Sitko during a bird ringing activity near a feeding box in a forest near Záhlinice (49.17 N, 17.30 E) on December 27–30, 2002. The morphology of the lesions corresponded to Figures 1 and 2. Two other trapped chaffinches were without papillomatous lesions.

Germany

One adult male chaffinch with papillomas extending over all four toes of the left leg was caught, ringed, and released on May 1, 2001 in Reifenstein (51.21 N, 10.21 E), Thuringia, central Germany by A. Goedecke. The appearance of the lesions was the same as shown in Figures 1 and 2. The tumour bled after releasing from the net.

Identification of papillomavirus

Electron microscopic examination of skin lesions of the dead chaffinch by the negative staining technique demonstrated typical papillomavirus virions composed of capsomeres arranged in icosahedral symmetry of the capside. The diameter of the virions measured approximately 52 to 55 nm. Only sporadic virions were found in the hyperkeratotic

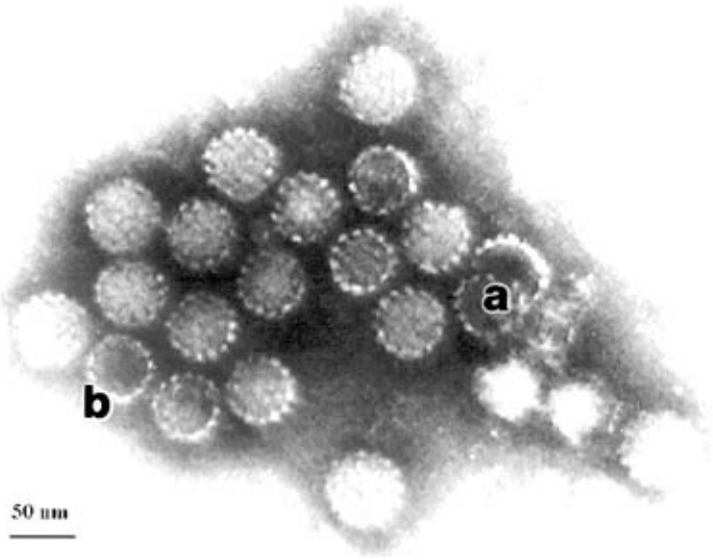

Figure 3. Cluster of papilloma virions. The virions are composed of morphological subunits (capsomeres) arranged in an icosahedral symmetry of the capsid. Virion size is 52–55 nm; a = destroyed virions, b = coreless virus particle. Negative staining with 2% water solution of ammonium molybdate, pH 7.0. Photographed by B. Šmíd and L. Valíček

surface layer, while large clusters were formed in the deeper layers (Figure 3). Both solitary destroyed (Figure 3a) and coreless particles with electron-dense core space (Figure 3b) were present in the aggregates.

DISCUSSION

Except for leg tumours in chaffinches, papillomatosis is rather rare in wild birds (Table 1). Leg papillomas in crows, lapwings, and starlings were described at the beginning of the 20th century. Rather exceptional were the findings of leg papillomas in a sedge warbler (*Acrocephalus schoenebaenus*) (Keymer and Blackmore, 1964), a waxwing (*Bombycilla garrulus*) (Lina, 1969), and in a brambling (Lina *et al.*, 1973). Many reports on findings of leg papillomas in chaffinches were published in the United Kingdom and the Netherlands and the disease occurs frequently also in Germany. Surprisingly, reports from other countries, such as Sweden, are sporadic only, although large populations of this species are living in all European countries.

Viral aetiology of typical papillomatous lesions in chaffinches had been suggested already before the causative agent was identified. Later reports indicate that members of the family *Papovaviridae* can infect several avian species. The well characterised papillomavirus FPV was demonstrated in leg papillomas of chaffinches (Jennings, 1968; Lina, 1970; Lina *et al.*, 1973). The agent was characterised as a papillomavirus by its size and density of virus particles, physical properties of viral DNA, and results of electrophoretic analyses of capsid proteins (Lina, 1970; Osterhaus *et al.*, 1977). The cloned FPV genome showed some homology with genomes of mammalian papillomaviruses in hybridisation studies and partial sequence analyses (Moreno-Lopez *et al.*, 1984). A comparison of restriction maps of a papillomavirus strain isolated from a chaffinch and a PePV papillomavirus strain isolated from a skin lesion in an African gray parrot did not reveal any common patterns (O'Banion *et al.*, 1992).

Infections by papillomavirus were described also in two greenfinches living in captivity for a long period together with canaries (*Serinus canaria*), a serine (*Serinus serinus*), a goldfinch (*Carduelis carduelis*), and a siskin (*Carduelis spinus*) of the family Fringillidae (Sironi and Gallazzi, 1992). Considering the fact that the infection was not transmitted to other bird species, Sironi and Gallazzi (1992) speculated on a strict host-specificity of such papillomatosis. The same authors described another case in which no transmission of papillomavirus was demonstrable between infected chaffinches and other members of the family Fringillidae (greenfinches, serines, siskins, and goldfinches) kept in the same premises. The question of among-species transmission of papillomavirus within the family Fringillidae, remains unanswered.

The primary diagnosis of papillomatosis in our birds was based on the finding of the typical leg lesions. In one case, the diagnosis was confirmed by the finding of particles typical of papillomavirus FPV as described by Osterhaus *et al.* (1977) and Sironi and Gallazzi (1992). This paper is apparently the first to describe cases of leg papillomatosis

in chaffinches in the Czech Republic and thus completes the current knowledge of geographical distribution of this agent. Nodular lesions in many species of wild birds including chaffinch are caused also with avipoxviruses (Bolte *et al.*, 1999). Avipoxvirus nodular lesions are usually on feather-free regions of the body, often on legs but they are compact and not papillomatous as it was in our cases.

Our cases were observed in various seasons and no conclusions as to possible seasonal dynamics can be made. Results of long-term observations of nine finches affected by leg papillomas indicate that the lesions can persist and grow for up to 2 years (Groth and Abs, 1967).

The mode of transmission, current prevalence of papillomavirus infections in chaffinches, bramblings, and other wild bird species, and effects of the infection on the fitness of the affected subpopulations are unknown and deserve continuing attention.

Acknowledgements

We thank our field co-workers A. Goedecke, V. Kovář, J. Sitko, M. Strnad, and J. Škopek for providing their data about lesions in chaffinches.

REFERENCES

Blackmore D.K., Keymer I.F. (1969): Cutaneous diseases of wild birds in Britain. Brit. Birds, *62*, 316–331.

Bolte A.L., Meurer J., Kaleta E.F. (1999): Avian host spectrum of avipoxviruses. Avian Pathol., *28*, 415–432.

Groth W., Abs K. (1967): Fusspapillomatose bei Buchfinken. Dtsch. Tierärztl. Wschr., *74*, 146–150.

Jennings A.R. (1959): Diseases of wild birds, fifth report. Bird Study, *6*, 19–22.

Jennings A.R. (1968): Tumors of free-living wild mammals and birds in Great Britain. Symp. Zool. Soc. London, *24*, 273–287.

Keymer I.F., Blackmore D.K. (1964): Diseases of the skin and soft parts of wild birds. Brit. Birds, *57*, 175–179.

Lina P.H.C. (1969): Tumours and wart-like excrescences on feet and legs of some wild birds. Ardea, *57*, 64–66.

Lina P.H.C. (1970): Viral particles in chaffinch papilloma cells. Ardea, *59*, 47–49.

Lina P.H.C., Noord M.J. van, Groot F.G. de (1973): Detection of virus in squamous papillomas of the wild bird species *Fringilla coelebs*. J. Natl. Cancer Inst., *50*, 567–571.

Moreno-Lopez J., Ahola H., Stenlund A., Osterhaus A., Pettersson U. (1984): Genome of an avian papillomavirus. J. Virol., *51*, 872–852.

Macdonald J.W. (1965): Chaffinches with papillomas. Brit. Birds, *58*, 346–347.

O'Banion M.K., Jacobson E.R., Sundberg J.P. (1992): Molecular cloning and partial characterization of a parrot papillomavirus. Intervirology, *33*, 91–96.

Osterhaus A.D.M.E., Ellens D.J., Horzinek M.C. (1977): Identification and characterization of a papillomavirus from birds (*Fringillidae*). Intervirology, *8*, 351–359.

Sironi G., Gallazzi D. (1992): Papillomavirus infection in greenfinches (*Carduelis chloris*). J. Vet. Med. B, *39*, 454–458.

Washington D. (1964): Unusual growths on feet of chaffinches. Brit. Birds, *57*, 184.

Zangger N., Müller M. (1990): Endemic papillomatosis of viral origin in water fowl of the Zoological Garden Basle. Schweiz. Arch. Tierheilkde, *132*, 483.

Received: 03–04–04
Accepted after corrections: 03–05–07

Corresponding Author

Prof. MVDr. Ivan Literák, CSc., University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Hygiene and Ecology, Palackého 1–3, 612 42 Brno, Czech Republic
Tel. +420 541 562 525, fax +420 541 211 229, e-mail: literaki@vfu.cz